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**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Application Number: 09/761,893
Filing Date: 1/17/2001
Appellant(s): HUNG et al.

Shih-Chieh Hung
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 4/24/2012.

(1) Grounds of Rejection to be Reviewed on Appeal

Every ground of rejection set forth in the Office action dated 11/17/2011 from which the appeal is taken is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading “WITHDRAWN REJECTIONS.” New grounds of rejection (if any) are provided under the subheading “NEW GROUNDS OF REJECTION.”

The following ground(s) of rejection are applicable to the appealed claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 4, 6, 9, 11, 34, 35 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Caplan et al** (US Patent No. 5,811,094; see the entire reference) in view of

Prockop et al (US Patent No. 7,374,937 B1, effective date March 14, 2000; see the entire reference) and **Matsui et al** (US Patent No. 4,871,674; see the entire reference).

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x 10³/cm² (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate

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into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48).

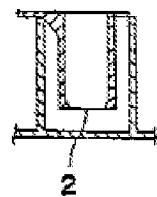
Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Prockop et al teach that RS cells can be separated from non-RS mesenchymal stem cells by ultrafiltration. Prockop et al teach that smaller RS cells will pass through an ultrafiltration membrane having appropriately sized pores, and such a membrane is a Millipore brand 10 micrometer isopore polycarbonate (plastic) membrane (e.g., column 39, line 60 to column 40, line 42).

Matsui et al teach culturing cells in a cell culture device comprising a cell culture insert comprising a membrane filter (2) on the bottom of the culture cell, which is composed of polycarbonate (e.g., column 2, lines 41-55; column 3, lines 5-18). The culture device is shown in Figure 8, which is reproduced below:

Fig. 8



It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan et al teach that mesenchymal stem cells adhere to plastic for culturing, and Caplan et al teach it is within the ordinary skill in the art to use a filter to remove fat cells and red blood cells from cells of bone marrow. Furthermore, Prockop et al teach the collection of mesenchymal stem cells on a filter of polycarbonate containing 10 micrometer pores, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter.

One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter, or other separation method, as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 4, 6, 9, 11, 34, 35 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Caplan et al** (US Patent No. 5,811,094; see the entire reference) in view of **Burkitt et al** (Wheater's Functional Histology (1993), page 60) and **Mussi et al** (US Patent No. 5,409,829; see the entire reference).

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x 10³/cm² (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the

mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Burkitt et al teach that red blood cells are 6.7-7.7 μm in diameter and nucleated cells have a diameter greater than 7.7 μm (page 60).

Mussi et al teach the introduction of a mixture of cells to be grown into a culture chamber in a suitable growth medium (e.g., column 2, lines 46-50). Mussi et al teach that the cells are grown in a culture insert contained within a well, where the insert is suspended in the well (e.g., paragraph bridging columns 3-4; Figure 4). The culture insert contains a membrane (20), which may be formed from a polymeric material such as polyethylene terephthalate, polycarbonate, and the like with open pores throughout (e.g., column 3, lines 50-53). Mussi et al teach that the pores are between about 0.2 to about 10 microns in diameter (e.g., column 3, lines 53-57).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells in medium into the culture insert of the culture device of Mussi et al because Caplan et al teach it is within the ordinary skill in the art to use a filter to remove red blood cells from cells of bone marrow aspirate and Mussi et al teach the use of a porous polycarbonate filter membrane, where the pore diameter can be about 0.2 to about 10 microns in diameter, and Burkitt et al teach that

red blood cells are the size which would pass through the filter of Mussi et al while nucleated mesenchymal stem cells of Caplan et al would be retained on top.

One would have been motivated to make such a modification in order to provide an enriched population of mesenchymal stem cells without the extra steps of using a column containing a filter, or other separation method, as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Mussi et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 4, 6, 9, 11, 34, 35 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Caplan et al** (US Patent No. 5,811,094; see the entire reference) in view of **Guirguis** (US Patent No. 5,077,012; see the entire reference) and **Matsui et al** (US Patent No. 4,871,674; see the entire reference).

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column

12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x 10³/cm² (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

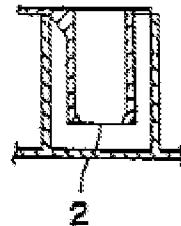
Guirguis teaches the removal of red blood cells from a body fluid using a membrane with a smooth flat surface which is ideal for the collection of atypical cells from all types of body

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fluids (e.g., column 3, lines 37-45; column 4). Guirguis et al teach that the membrane has a preferred pore size of 2 microns or less (e.g., column 4, lines 14-19). Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity (e.g., column 4, lines 43-64).

Matsui et al teach culturing cells in a cell culture device comprising a cell culture insert comprising a membrane filter (2) on the bottom of the culture cell, which is composed of polycarbonate (e.g., column 2, lines 41-55; column 3, lines 5-18). The culture device is shown in Figure 8, which is reproduced below:

Fig. 8



It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan et al teach it is within the ordinary skill in the art to culture mesenchymal stem cells on plastic and teach the use a filter to remove fat cells and red blood cells from cells of bone marrow. Furthermore, Guirguis teaches the use of a polycarbonate membrane for the removal of red blood cells from a body fluid, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter.

One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al. Further, one would have been motivated to use the polycarbonate (plastic) filter in place of the Leukosorb filter taught by Caplan et al, because Caplan et al teach that mesenchymal stem cells become selectively attached to plastic in DMEM containing 10% FBS and 1 g/L of glucose or complete medium, and Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity of the polycarbonate. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

(2) Response to Argument

Appeal of restriction requirement between Groups I and II as set forth in the Office action mailed 8/7/2001

At page 3, section (vi), Appellant requests the Board to reverse the withdrawing from consideration of claims 43-45. Thus, Appellant is requesting the Board to reverse the restriction requirement between Groups I and II as set forth in the Office action mailed 8/7/2001.

Applicant elected Group I without traverse in the reply filed 9/4/2001. Thus, Applicant has not preserved the right of petition review of the requirement by the Director. This issue is not subject to appeal to the Board of Patent Appeals and Interferences. See 37 C.F.R. § 1.144.

Rejection of claims 1, 4, 6, 9, 11, 34, 35 and 38 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094) in view of Prockop et al (US Patent No. 7,374,937 B1) and Matsui et al (US Patent No. 4,871,674)

The brief asserts that the rejection fails to explain why a person of ordinary skill in the relevant field would combine the prior art elements in the manner claimed.

This argument if not found persuasive. The rationale is provided in the last two paragraphs of the rejection of record. It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan et al teach that mesenchymal stem cells adhere to plastic for culturing, and Caplan et al teach it is within the ordinary skill in the art to use a filter to remove fat cells and red blood cells from cells of bone marrow. Furthermore, Prockop et al teach the collection of mesenchymal stem cells on a filter of polycarbonate containing 10 micrometer pores, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter. One of ordinary skill in the art at the time the invention was made would have recognized that the dish of Matsui et al contains a membrane suitable for the culture of mesenchymal stem cells and suitable for the passage of red blood cells. Further evidence is provided by Guirguis (US Patent No. 5,077,012) who teaches that

polycarbonate membranes are suitable for passage of red blood cells without clogging while retaining nucleated cells (e.g., column 4, lines 14-19 and 43-55). One in the art would have recognized that the apparatus of Matsui et al has the characteristics necessary for separation of red blood cells from mesenchymal stem cells and for culture of mesenchymal stem cells. One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter, or other separation method, as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al.

The brief asserts that there was no reasonable expectation of success. Each reason provided by Appellant will be addressed in turn.

First, the brief cites Prockop's disclosure (US Patent No. 7,374,937), which states that "this review of the literature demonstrates that transplantation of MSCs have significant therapeutic and gene transfer uses. However, prior art methods for isolating MSCs and inducing their proliferation have practical limitations, including the extent of population expansion that can be achieved using prior art methods. There remains a critical need for methods of reliably inducing significant proliferation of MSCs in culture without inducing differentiation of the MSCs as they proliferate."

This argument is not found persuasive. The complete passage found at column 5, lines 19-28 is reproduced here:

This review of the literature demonstrates that transplantation of MSCs have significant therapeutic and gene transfer uses. However, prior art methods for isolating MSCs and inducing their proliferation have practical limitations, including the extent of population expansion that can be achieved using prior art

methods. There remains a critical need for methods of reliably inducing significant proliferation of MSCs in culture without inducing differentiation of the MSCs as they proliferate. **The present invention satisfies this need.** (Emphasis added.)

Prockop solved the difficulties in the art noted by Appellant, and one would have had a reasonable expectation of success.

Second, the brief cites a number of references, which teach differentiation of MSCs performed using cultures of MSCs that have been isolated primarily by virtue of the MSCs tight adherence to tissue culture dishes. The references cited are as follows:

- (1) Friedenstein et al. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Experimental Hematology*, Vol. 4, pages 267-274, September 1976.
- (2) Friedenstein et al. Bone marrow osteogenic stem cells: *in vitro* cultivation and transplantation in diffusion chambers. *Cell and Tissue Kinetics*. Vol. 20, No. 3, pages 263-272, May 1987.
- (3) Long et al. Regulation of human bone marrow-derived osteoprogenitor cells by osteogenic growth factors. *Journal of Clinical Investigation*, Vol. 95, pages 881-887, February 1995 (of record).
- (4) Simmons et al. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood*, Vol. 78, pages 55-62, July 1991.
- (5) Waller et al. The “common stem cell” hypothesis reevaluated: human fetal bone marrow contains separate populations of hematopoietic and stromal progenitors. *Blood*, Vol. 85, pages 2422-2435, May 1995.
- (6) Rickard et al. Isolation and characterization of osteoblast precursor cells from human bone marrow. *Journal of Bone and Mineral Research*, Vol. 11, No. 3, pages 312-324, 1996.
- (7) Joyner et al. Identification and enrichment of human osteoprogenitor cells by using differentiation stage-specific monoclonal antibodies. *Bone*, Vol. 21, No. 1, pages 1-6, July 1997.

The brief asserts that none of these protocols disclosed by the above references has gained wide acceptance, and the protocols have been primarily designed to isolate osteoblast precursors. The brief asserts that use of these protocols has not been investigated to determine if they yield cells that are truly multipotential.

Copies of references (1), (2) and (4)-(7) have not been provided by Applicant. Thus, copies of the references have been obtained and attached hereto. Appellant's argument is similar to the background information provided by Caplan at the paragraph bridging columns 4-5:

Despite the great interest in examining the biology of MSCs and their potential use for therapy, there is still no generally accepted protocol for isolating and expanding MSCs in culture. Most experiments relating to differentiation of MSCs have been performed using cultures of MSCs that have been isolated primarily by virtue of the MSCs tight adherence to tissue culture dishes, as described (Friedenstein et al., 1976, Exp. Hematol. 4:267-274; Friedenstein et al., 1987, Cell Tissue Kinet. 20:263-272). Others have attempted to prepare more homogenous MSC populations (e.g. Long et al., 1995, J. Clin. Invest. 95:881-887; Simmons et al., 1991, Blood 78, 55-62; Waller et al., 1995, Blood 85:2422-2435; Rickard et al., 1996, J. Bone Miner. Res. 11:312-324; Joyner et al., 1997, Bone 21:1-6). However, none of these protocols has gained wide acceptance. In addition, these protocols have been primarily designed to isolate osteoblast precursors. Use of these protocols has not been investigated to determine if they yield cells that are truly multipotential.

At column 5, lines 19-28, Caplan go on to state the following:

This review of the literature demonstrates that transplantation of MSCs have significant therapeutic and gene transfer uses. However, prior art methods for isolating MSCs and inducing their proliferation have practical limitations, including the extent of population expansion that can be achieved using prior art methods. There remains a critical need for methods of reliably inducing significant proliferation of MSCs in culture without inducing differentiation of the MSCs as they proliferate. **The present invention satisfies this need.** (Emphasis added.)

At column 10, lines 61-67, Caplan state the following:

In the MSC culture/expansion method of the invention, MSCs retain their multipotentiality (i.e., their capacity to differentiate into one of various cell types, such as osteoblasts, adipocytes, and the like). MSCs expanded using the method of the invention retain their ability to differentiate to a greater extent (i.e., in greater proportion) than do MSCs expanded or cultured using prior art methods.

Caplan solved the difficulties in the art noted by Appellant, and one would have had a reasonable expectation of success. The cited references do not negate the teachings of Caplan.

Third, the brief cites Kato et al (US Patent Application 2005/0013804, filing date 9/12/2001), which states “the conventional culture methods however cannot produce sufficient amounts of mesenchymal stem cells because the proliferation of said stem cells stops or becomes extremely slow around 15th generation.

This argument is not found persuasive. In Example 2, Prockop et al state the following:

The data disclosed in this example demonstrate that plating MSCs at a low density (e.g., between about 0.5 to about 10 cells per square centimeter) increases, relative to prior art culture techniques, the number of population doublings which the MSCs can undergo **without also undergoing differentiation**. As a result, differentiable MSCs can be expanded in culture to a far greater extent than they can using prior art culture/expansion methods. (Column 27, lines 29-37). (Emphasis added.)

Prockop et al state the following at column 29, lines 27-24:

Moreover, the **number of cell doublings is increased three-fold relative to prior art culture conditions**, as indicated in FIG. 9. Therefore, the MSC culture/expansion method described in this example, allows greater number of MSCs to be generated, and including a **greater percentage of differentiable (i.e., multipotential) cells than do prior art culture methods**. (Emphasis added.)

Using the "low density" method, Prockop et al teach the following at column 16, lines 49-61:

There is no theoretical limit to the number of rounds of expansion and harvest that can be performed. However, it is recognized that because each expansion/harvest cycle will significantly increase the number of MSCs available (i.e., by 10-fold, 100-fold, or more), a geometrically increasing amount of growth medium and growth surface will be required during sequential expansion/harvest cycles if all expanded MSCs are to be further expanded. Thus, it is recognized that for most applications, no more than about 10 cycles of expansion and harvest will normally be necessary, and as few as 1, 2, 3 or 4 cycles will be sufficient for many applications (e.g., cell therapy or gene therapy). (Emphasis added.)

Thus, Prockop et al teach culture methods that can produce sufficient amounts of mesenchymal stem cells. In response to applicant's argument that the references fail to show

certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., producing a particular amount of stem cells or culture for a particular number of population doublings or generations) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Although claim 45 recites "wherein the mesenchymal stem cells proliferate without differentiation and reach confluence after 12 passages," this claim is withdrawn from examination as being drawn to a non-elected invention. The present specification states, "The cells maintain a normal proliferation and undifferentiation status during culture expansion even at passage 12." See page 14, lines 23-25. The present specification does not provide evidence that the cells re-plated at 4 x 10³-10⁴ cells/cm² for 12 passages are able to differentiate to adipose or cartilage as presently claimed in claim 9. The specification teaches differentiation of the MSCs 14 days following the first passage (Example 4). In this aspect, the specification does not support nonobviousness of the claimed method, and Applicant has not provided objective evidence demonstrating an unexpected superior result obtained by the claimed method as compared to the closest prior art.

Fourth, the brief cites Lange et al (US Patent Application 2007/0160583, filing date 8/6/2004), which states "from the results known to prior art, it is clear that sprouting of less mitotic cells cannot be prevented with any separation method. Rather the cultivation conditions play a role here. What is needed here is careful selection of the population with most evident proliferation features."

This argument is not found persuasive. It is unclear how these statements relate to the claimed invention or to the teachings of the references cited in the rejection. As discussed above, both Caplan and Prockop provide methods of successfully culturing multipotent MSCs.

Fifth, the brief cites Lin et al (US Patent Application Publication 2007/0128722, filing date 12/5/2005), which states "due to the reason that adult stem cells are rare in adult tissues and it is difficult to expand their numbers in cell culture, methods of proliferating adult stem cells in culture are sought."

This argument is not found persuasive. As discussed above, both Caplan and Prockop provide methods of successfully culturing and proliferating multipotent MSCs.

Considering all the art on the record, one would have had a reasonable expectation of success in combining the teachings of Caplan, Prockop and Matsui in the manner discussed in the rejection of record.

The brief cites Barker et al (US Patent No. 5,652,142) as criticizing Matsui's culture insert, because "gases may not be exchanged sufficiently because the area between the sidewall of the insert and culture plate is too small." (Column 1, lines 37-39).

This argument is not found persuasive. Barker teaches that the cell culture inserts and devices described in US Patent No. 4,871,674 to Matsui et al are conventional cell culture inserts and devices (column 1, lines 27-31). Thus, they are generally used in the art, and the use of such inserts and devices would have been predictable. It is more likely than not that the culture dishes of Matsui et al are suitable for the culture of cells on the insert. Applicant has not provided evidence that the presently claimed invention requires a larger area between the side-wall of the insert and the culture plate, and the claims do not require a particular distance between the upper

plate and a wall of the culture device containing the upper plate. Even if the area between the sidewall of the insert and the culture plate was found to be too small for culturing mesenchymal stem cells on the porous insert, one would have increased the area to prevent or restrict the outer surface of the cell culture insert sidewall from moving close to the inner wall of the well of the tissue culture vessel in which it is placed so that capillary action of the fluid in the well is minimized as taught by Barker (e.g., column 2, lines 35-40; column 3, lines 48-64). Thus, the prior art presents a possible problem with the culture dish of Matsui et al and provides a solution to overcome the problem should it arise in a particular application of a culture insert.

The brief notes that the Matsui patent issued on 10/3/1989, Caplan's patent application was filed on 4/11/1995 and issued on 9/22/1998, and Prockop's patent was filed on 10/25/2000 (effective date 3/14/2000). The brief notes that the present application was filed on 1/17/2001 and claims foreign priority to a document filed 10/17/2000. The brief asserts that these dates do not support the assertion that it would have been obvious to combine the teachings of Matsui, Caplan and Prockop. The brief asserts that if it had been obvious, Prockop would have combined the teachings of Matsui and Caplan to reach the claimed method. The brief notes that Prockop has been a well-known mesenchymal stem cell research but did not reach the claimed method.

In response to applicant's argument based upon the age of the references, contentions that the reference patents are old are not impressive absent a showing that the art tried and failed to solve the same problem notwithstanding its presumed knowledge of the references. See *In re Wright*, 569 F.2d 1124, 193 USPQ 332 (CCPA 1977). Applicant has not provided evidence that Prockop tried and failed to solve the same problem despite knowledge of the older references.

Rejection of claims 1, 4, 6, 9, 11, 34, 35 and 38 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094) in view of Burkitt et al (Wheater's Functional Histology (1993), page 60) and Mussi et al (US Patent No. 5,409,829)

It is noted that the brief refers to the rejection as being over Caplan in view of Burkitt and Matsui. However, the rejection of record is over Caplan in view of Burkitt et al and Mussi.

The brief asserts that the rejection fails to explain why a person of ordinary skill in the relevant field would combine the prior art elements in the manner claimed.

This argument if not found persuasive. The rationale is provided in the last two paragraphs of the rejection of record. It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells in medium into the culture insert of the culture device of Mussi et al because Caplan et al teach it is within the ordinary skill in the art to use a filter to remove red blood cells from cells of bone marrow aspirate and Mussi et al teach the use of a porous polycarbonate filter membrane, where the pore diameter can be about 0.2 to about 10 microns in diameter, and Burkitt et al teach that red blood cells are the size which would pass through the filter of Mussi et al while nucleated mesenchymal stem cells of Caplan et al would be retained on top. One of ordinary skill in the art at the time the invention was made would have recognized that the dish of Mussi et al contains a membrane suitable for the culture of mesenchymal stem cells and suitable for the passage of red blood cells. Further evidence is provided by Guirguis (US Patent No. 5,077,012) who teaches that polycarbonate membranes are suitable for passage

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of red blood cells without clogging while retaining nucleated cells (e.g., column 4, lines 14-19 and 43-55). One in the art would have recognized that the apparatus of Mussi et al has the characteristics necessary for separation of red blood cells from mesenchymal cells and for culture of mesenchymal cells. One would have been motivated to make such a modification in order to provide an enriched population of mesenchymal stem cells without the extra steps of using a column containing a filter, or other separation method, as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Mussi et al.

The brief asserts that a person of ordinary skill in the relevant field would have difficulty using Matsui's culture device.

This argument is not found persuasive. The rejection of record does not rely on the teachings of Matsui. The combined teachings of Caplan, Burkitt and Mussi result in using Mussi's culture device.

The brief asserts that there was no reasonable expectation of success. The brief states that Caplan clearly disclosed that "as a whole, bone marrow is a complex tissue comprised of hematopoietic stem cells, red and white blood cells and their precursors, mesenchymal stem cells, stromal cells and their precursors, and a group of cells including fibroblasts, reticulocytes, adipocytes, and endothelial cells which form a connective tissue network called "stroma". (Column 7, line 12-16, US Patent 5811094) The brief notes that the red blood cell taught by Burkitt is only one of the components in bone marrow. The brief notes that after the removal of red blood cells from aspirated marrow, a pure population of mesenchymal stem cells has not

been isolated. The brief assert that a person of ordinary skill in the relevant field would not modify Caplan's method in view of Burkitt's teaching.

This argument is not found persuasive. Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34). To provide an enriched population of mesenchymal stem cells, one would have been motivated to remove red blood cells. Further, Caplan teach "Compositions having greater than 95%, usually greater than 98% of human mesenchymal stem cells can be achieved using the previously described technique for isolation, purification and culture expansion of MSCs" (column 6, lines 29-32), where the previously described technique

includes isolation and purification of human MSCs from tissue, such as bone marrow, by their selective attachment, termed "adherence" to substrates when cultured in a specific medium (column 6, lines 13-28). Thus, it is not unexpected that MSCs can be purified from bone marrow aspirate by culturing the cells on a plastic culture insert to which the mesenchymal stem cells adhere, and changing the medium to remove non-adherent cells. One would have recognized that combining selective adherence with removal of red blood cells by the culture insert of Mussi et al would have resulted in a population of MSCs that are at least 95% pure or at least 98% pure. However, it is noted that the present claims do not require a particular level of purity to be achieved by the claimed method. Although claim 43 recites "cell populations having greater than 98% homogeneous mesenchymal stem cells," this claim is withdrawn as being drawn to a non-elected invention.

Rejection of claims 1, 4, 6, 9, 11, 34, 35 and 38 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094) in view of Guirguis (US Patent No. 5,077,012) and Matsui et al (US Patent No. 4,871,674)

The brief asserts that the rejection fails to explain why a person of ordinary skill in the relevant field would combine the prior art elements in the manner claimed.

This argument if not found persuasive. The rationale is provided in the last two paragraphs of the rejection of record. It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan

et al teach it is within the ordinary skill in the art to culture mesenchymal stem cells on plastic and teach the use a filter to remove fat cells and red blood cells from cells of bone marrow. Furthermore, Guirguis teaches the use of a polycarbonate membrane for the removal of red blood cells from a body fluid, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter. One of ordinary skill in the art at the time the invention was made would have recognized that the apparatus of Matsui et al has the characteristics necessary for separation of red blood cells from mesenchymal stem cells and for culture of mesenchymal stem cells. One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al. Further, one would have been motivated to use the polycarbonate (plastic) filter in place of the Leukosorb filter taught by Caplan et al, because Caplan et al teach that mesenchymal stem cells become selectively attached to plastic in DMEM containing 10% FBS and 1 g/L of glucose or complete medium, and Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity of the polycarbonate.

The brief asserts that a person of ordinary skill in the relevant field would have difficulty in using Matsui's culture device. Specific reasons for this difficulty are not provided in the arguments to the combined teachings of Caplan, Guirguis and Matsui. In arguing the combination of Caplan, Prockop and Matsui, the brief cites Barker et al (US Patent No.

5,652,142) as criticizing Matsui's culture insert, because "gases may not be exchanged sufficiently because the area between the sidewall of the insert and culture plate is too small." (Column 1, lines 37-39).

This argument is not found persuasive. Barker teaches that the cell culture inserts and devices described in US Patent No. 4,871,674 to Matsui et al are conventional cell culture inserts and devices (column 1, lines 27-31). Thus, they are generally used in the art, and the use of such inserts and devices would have been predictable. It is more likely than not that the culture dishes of Matsui et al are suitable for the culture of cells on the insert. Applicant has not provided evidence that the presently claimed invention requires a larger area between the side-wall of the insert and the culture plate, and the claims do not require a particular distance between the upper plate and a wall of the culture device containing the upper plate. Even if the area between the sidewall of the insert and the culture plate was found to be too small for culturing mesenchymal stem cells on the porous insert, one would have increased the area to prevent or restrict the outer surface of the cell culture insert sidewall from moving close to the inner wall of the well of the tissue culture vessel in which it is placed so that capillary action of the fluid in the well is minimized as taught by Barker (e.g., column 2, lines 35-40; column 3, lines 48-64). Thus, the prior art presents a possible problem with the culture dish of Matsui et al and provides a solution to overcome the problem should it arise in a particular application of a culture insert.

The brief asserts that a person of ordinary skill in the relevant field would not modify Caplan's method in view of Guirguis' teaching, because Guirguis disclosed "an apparatus for collecting biological fluids and holding samples taken from a biological fluid for qualitative and quantitative testing." (Abstract, US patent 5077012). The brief asserts that Guirguis' apparatus

was "for detecting disease markers both for screening as well as for a reference laboratory setting." (Column 1 line 15 to line 17).

In response to applicant's argument that Guirguis is nonanalogous art, it has been held that a prior art reference must either be in the field of applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the applicant was concerned, in order to be relied upon as a basis for rejection of the claimed invention. See *In re Oetiker*, 977 F.2d 1443, 24 USPQ2d 1443 (Fed. Cir. 1992). In this case, Caplan teaches the removal of red blood cells from bone marrow aspirate (body fluid) for the purpose of providing purified MSCs (column 8, lines 20-44; column 11, lines 35-62). Guirguis teaches the removal of red blood cells from a body fluid using a membrane with a smooth flat surface, which is ideal for the collection of atypical cells from all types of body fluids (e.g., column 3, lines 37-45; column 4). Guirguis teaches that the membrane has a preferred pore size of 2 microns or less (e.g., column 4, lines 14-19). Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity (e.g., column 4, lines 43-64). Thus, one would have recognized that the membrane of Guirguis could be used to remove red blood cells from nucleated cells, and Caplan is concerned with removing red blood cells from nucleated MSCs. The references are both in the same field of endeavor related to cell separation from a fluid, where cell separation involves the removal of red blood cells from a population of nucleated cells.

The brief notes that Prockop stated that "despite the great interest in examining the biology of MSCs and their potential use for therapy, there is still no generally accepted protocol

for isolating and expanding MSCs in culture." This brief notes that the present specification discloses that "in one preferred embodiment of the present invention, the isolated mesenchymal stem cells proliferate without differentiation and reach confluence even after 12 passages: The cell populations having greater than 98% homogeneous MSCs are obtained in accordance with the method of the present invention." [0031] The response asserts that this result is unexpected, and the unexpected result is supported by post-filing art (Kato et al 2005/0013804, filing date 9/12/2001), which mentioned that "The conventional culture methods however cannot produce sufficient amounts of mesenchymal stem cells because the proliferation of said stem cells stops or becomes extremely slow around 15th generation."

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., producing a particular amount of stem cells or culture for a particular number of population doublings or generations) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Although claim 45 recites "wherein the mesenchymal stem cells proliferate without differentiation and reach confluence after 12 passages," this claim is withdrawn from examination as being drawn to a non-elected invention. The present specification states, "The cells maintain a normal proliferation and undifferentiation status during culture expansion even at passage 12." See page 14, lines 23-25. The present specification does not provide evidence that the cells obtained by the claimed method and replated at 4×10^3 - 10^4 cells/cm² for 12 passages are able to differentiate to adipose or cartilage as presently claimed in claim 9.

Prockop et al (US Patent No. 7,374,937) teaches the propagation of human bone marrow MSCs with re-plating at a density of 5×10^3 cells/cm² with growth to near confluence (e.g., column 22, lines 19-33). This is referred to as High Density Plating (e.g., paragraph bridging columns 21-22). Some MSC samples only proliferated for four population doublings, and other proliferated over 15 cell doublings from frozen cell stocks (e.g., paragraph bridging columns 23-24; Fig. 1). Differentiation assays were carried out with "late passage MSCs (e.g., passage 12)." The "late passage MSCs" retained the ability to differentiate to osteoblasts; however, the MSCs failed to differentiate into adipocytes (e.g., column 24, lines 57-67).

The specification teaches high density plating and differentiation of the MSCs 14 days following the first passage (Example 4). The specification does not provide evidence that the MSCs could be cultured for 12 or 15 population doublings and retain the ability to differentiate to adipose or cartilage. Thus, the present specification does not provide evidence of unexpected results of the claimed method. Kato et al (2005/0013804) does not provide evidence of unexpected results of the claimed method, because Kato et al do not carry out the claimed method.

Caplan teach "Compositions having greater than 95%, usually greater than 98% of human mesenchymal stem cells can be achieved using the previously described technique for isolation, purification and culture expansion of MSCs" (column 6, lines 29-32), where the previously described technique includes isolation and purification of human MSCs from tissue, such as bone marrow, by their selective attachment, termed "adherence" to substrates when cultured in a specific medium (column 6, lines 13-28). Thus, it is not unexpected that MSCs can be purified from bone marrow aspirate by culturing the cells on a plastic culture insert to which the

mesenchymal stem cells adhere, and changing the medium to remove non-adherent cells. One would have recognized that combining selective adherence with removal of red blood cells by the culture insert of Matsui et al would have resulted in a population of MSCs that are at least 95% pure or at least 98% pure.

Remarks regarding the Pre-Appeal Brief Review Summary

In response to the statement that "Prockop et al teach a method that allows a greater number of MSCs to be generated, including a greater percentage of differentiable (i.e., multipotential cells) than prior art culture methods, one would have expected the culture conditions taught by Prockop et al to provide culture to confluence for 12 passages without differentiation," the brief newly cites Tsai et al (Blood, Vol. 117, No. 2, pages 459-469, 2011) and Leong et al (Stem Cells, Vol. 22, No. 6, pages 1123-1125, 2004) as providing evidence that low density culture cannot be extended for up to 6-7 passages. The brief asserts that the cells undergo senescence, and one would not have expected the culture conditions taught by Prockop to provide culture to confluence for 12 passages without differentiation.

Copies of Tsai et al and Leong et al have not been provided. Thus, copies have been obtained and attached hereto.

These arguments have not been found persuasive.

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., culture to confluence for 12 passages) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the

claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Although claim 45 recites “wherein the mesenchymal stem cells proliferate without differentiation and reach confluence after 12 passages,” this claim is withdrawn from examination as being drawn to a non-elected invention.

Tsai et al teach that an increase in seeding density decreases expansion efficiency (paragraph bridging pages 461-462). Tsai et al state, “Increase in cell density number by up to 170- to 250-fold for each passage was seen for low-density (approximately 50 cells/cm²) culture, whereas high density (approximately 1000-4000 cells/cm²) culture showed a fivefold increase for each week.” (paragraph bridging pages 461-462). Tsai et al teach that continuous low-density culture resulted in a decrease in expansion efficiency, and this decrease could be prevented using hypoxic culture (1% O₂) (e.g., paragraph bridging pages 462-463). Tsai et al teach that cells grown under normoxic conditions began to cease proliferation and were difficult to subculture after passage 6 to 7. However, Prockop teaches that some MSC samples in high density culture only proliferated for four population doublings, and others proliferated over 15 cell doublings from frozen cell stocks (e.g., paragraph bridging columns 23-24; Fig. 1). In Example 2, Prockop teaches that the number of cell doublings increased three-fold relative to prior art high density cultures. Thus, one would expect to be able to culture the cells for 12 passages even if it became difficult after 6 or 7. Furthermore, the present claims do not require hypoxic conditions for extending the passage of the cells, and the present specification does not disclose such a modification to culture conditions to achieve this benefit.

The teachings of Leong do not provide evidence of unexpected results for the claimed invention. Leong discusses one embodiment of the presently claimed invention where Percoll

gradient separated bone marrow cells are plated on double-decker culture plates (Transwell, Corning, NY) where the top plate was fabricated with 3- μ m pores, and size-sieved (SS) cells on the upper plate were described to be fibroblastic in morphology and large in size, while the lower plate (LP) cells were small and polygonal (e.g., page 1123, left column, 2nd paragraph). Leong states the following regarding this embodiment of the presently claimed invention:

However, it would be more convincing to the scientific communities of stem cell and tissue engineering if the LP cells were also characterized and shown not to possess the same differentiation potential and characteristics. This would be important before one can arrive at the conclusion that successful “sieving” of stem cells had occurred. The “stem-ness” of the separated cell population subsets (SS,LP) would directly correlate with the efficacy of the proposed strategy. The greater the efficacy, the more localized stemness would be on SS cells and the less on LP cells. Consequently, this would mean that the SS to LP data justify and reflect the efficacy of the procedure. Reporting SS cells as having stem cell characteristics may not be adequate because without sieve separation, the multipotency of bone marrow– derived stem cells have already been reported [6–10], thus leading us to expect some degree of stem cell characteristics with the SS cells anyway. Confirming the stem-ness of a stem cell population must precede its characterization. Without such confirmation, any conclusions arising from the characteristics of the purported stem cell population would be incomplete, and the derived clinical applications would have profound repercussion throughout the field. Ideally, research should assess stem-ness in terms of cellular self-renewal ability—the differentiation of in vitro and in vivo single-cell clones into cell types of the tissue of origin and at least one other different tissue cell type [11].

At the paragraph bridging the columns of page 1124, Leong states the following:

Additionally, to further validate the success of the sieve strategy in separating stem cells from other non-stem cells in the population, one might also investigate the presence and characteristics of non-stem cells, in particular, in the SS fraction. It is challenging to comprehend this method as a purification strategy for marrow stem cells as other cells found in the marrow are all too large for the “3- μ m” pores; examples include lymphocytes (9–15 μ m), granulocytes (12–15 μ m), nucleated red cells (6–12 μ m), and monocytes (16–20 μ m) [14]. Based on the pore size of 3 μ m of the upper plate, these contaminating cells would still be retained in the SS fraction but would be unseparated from the stem cell population. Furthermore, based on Table 1 presented in the original Hung et al. paper, it could be argued that there might be still contamination of myeloid cells (CD38+) [15], leukocytes (CD50+) [16], and hematopoietic progenitors (HLA-

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DRdim+, CD90+) [17] cells in the SS fraction. However, these findings were not discussed in sufficient depth by the authors. Based on the current literature [11, 18], the absence of these cells in a pure stem cell culture is a *conditio sine qua non* to confirm efficacious purification of stem cells out of inhomogeneous cell population. In any proposed stem cell isolation process, the efficacy of the separation procedure can be demonstrated when the proportion of these “contaminating” cells is minimized or even depleted. Thus Hung et al. may need to show in future studies that there is a significantly lower proportion of contaminating marrow cell types among the SS cells compared to the LP cells and the unseparated cell population. The efficacy of this stem cell separation procedure can therefore be demonstrated if a significantly lower proportion of contaminating cells is observed. With sufficiently low contaminating cells, the additional step of depleting such “contaminating” cells via cell sorters (using cell surface markers like STRO-1 and CD105) may then be avoided. In addition, **the use of 3 μ m might filter out residual amounts of red blood cells (RBCs) and platelets (2–4 μ m) that are not entirely removed by the Percoll fractionation method.** Though RBCs are 7.5 μ m in size, they are able to deform and squeeze through 2 μ m capillaries (cellular sizes referenced from [14]). (Emphasis added.)

Thus, Leong is critical of the assertions that the one embodiment of the presently claimed method provides an improvement over prior art methods. The scientific community has not been convinced of the assertions of unexpected improvements resulting from one embodiment of the claimed method. The teachings of Leong do not support the nonobviousness of the presently claimed method. The statements of Leong provide support to the rationale that one would have expected the prior art culture dishes containing a plate with pores to be capable of filtering out red blood cells.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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